continuation question #9

Phenotypes will be obtained on the mothers, and where possible, the fathers of non-MM newborns. A detailed reproductive history will be obtained and compared with that of a randomly selected control population of mothers of MM newborns. This will include the number of spontaneous abortions, induced abortions, living children and total pregnancies. The history will also include use of contraceptive techniques if any. Pending analysis of the data it may not be appropriate to offer family counseling to the non-MM group.

An additional laboratory study will be a comparison of the temperature stability of MM phenotypes in newborns with those of children. Sialic acid levels using the assay of Warren[5] and sialyltransferase levels as determined by Kuhlenschmidt et al[6] will be measured in each serum. Our previous studies suggest that the biosynthesis of alpha-1-antitrypsin may be incomplete at birth. The MM pattern of newborns, in our study, has a more cathodal mobility which is different from that seen in children. The sialic acid component may be the basis for phenotypic distinction, as suggested by Cox[7] and by Bell and Carrell,[8] and could contribute to the variation in the MM pattern typical of newborn infants.

question #12

Dr. Hugh E. Evans REDACTED REDACTED He graduated from Columbia College, R cum laude, and Downstate Medical School, R His Internship and Residency were at Johns Hopkins Hospital, 1958-60, 1962-63. He was a Clinical Associate in the National Institute of Allergy and Infectious Diseases, N.I.H., Bethesda, Maryland, 1960-62. He was Associate Director of Pediatrics, Harlem Hospital Center and Associate Clinical Professor of Pediatrics, Columbia University, 1966-73. Presently he is Professor of Pediatrics, Downstate Medical Center and Director of Pediatrics, Jewish Hospital and Medical Center of Brooklyn, Memberships include REDACTED REDACTED REDACTED interests include the role of alpha-1-antitrypsin deficiency in neonatal lung disease and factors influencing the neonatal bacterial flora. He is senior author of the textbook, "Perinatal Medicine," which is in press for October, 1975.

Dr. Yong Ho Shin REDACTED He graduated from Pusan National University in R and from the Pusan National University School of Medicine in R He spent 4 years as a physician in the South Korean Army, the last 2 of which were in a Tuberculosis Hospital in Masan, Korea. His Internship, in this country was at Christ Hospital, Jersey City, New Jersey (1969) and he was a first year Resident in Martland Hospital, Newark, New Jersey in 1970. Following this he was a Senior Resident and a Fellow in Pulmonary Disease at Harlem Hospital Center, 1971-June 1973. He completed his training in Pulmonary Disease at the Jewish Hospital and Medical Center of Brooklyn in June 1974. He is a full-time Attending, in charge of Pulmonary Disease at JHMCB, and a Clinical Instructor in Pediatrics at Downstate Medical Center. He has been an active participant in the studies outlined.

14. First year budget:

A. Salaries (give names or state "to be recruited") Professional (give % time of investigator(s) even if no salary requested)	% time	Amount
Dr. Hugh E. Evans	25%	R
Dr. Yong Ho Shin	50%	R
Technical		
Miss Nora Formaini	90%	R
Miss Lynn Perrott	90%	R
	Sub-Total for A	REDACTED

B. Consumable supplies (by major categories)

1. Material for isoelectric focusing.	1,295
2. Material for crossed antigen-antibody electrophoresis.	2,480
3. Material for antitrypsin activity testing.	170
4. Material for radial immunodiffusion.	400
(See detailed list appended) Sub-Total for B	$4,345

C. Other expenses (itemize)

Sub-Total for C	
Running Total of A + B + C	30,745

D. Permanent equipment (itemize)

Sub-Total for D	
E. Indirect costs (15% of A+B+C) E	4,612
Total request	$35,357

15. Estimated future requirements:

	Salaries	Consumable Suppl.	Other Expenses	Permanent Equip.	Indirect Costs	Total
Year 2	REDACTED	$4,649			$4,935	$37,832
Year 3						

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question #9 (continued)

References for Laboratory Methods

1. Fagerhol, M.K. The Pi-system: Genetic variants of serum alpha-1-antitrypsin. Ser. Haematal 1: 153-161, 1968

2. Fagerhol, M.K. and Laurell, C-B. The polymorphism of "pre-albumins" and alpha-1-antitrypsin in human sera. Clin. Chem. Acta 16:199, 1967

3. Mancini, M., Carbonara, A. and Heremans, F. Immunochemical quantitation of antigens by single radial immunodiffusion. Immun. Chem. 2:234, 1965

4. Erlanger, B.F., Kokowsky, N and Cohen W. The preparation and properties of two new chromogenic substrates or trypsin. Arch. Biochem. 95:271, 1961

5. Warren, L. The thiobarbituric acid assay of sialic acids. J. Biol. Chem. 8:1971-1975, 1959

6. Kuhlenschmidt, M.S. et al. Demonstration of sialyltransferase deficiency in the serum of a patient with alpha-1-antitrypsin deficiency and hapatic cirrhosis. Lab. Inves. 4:413-419, 1974

7. Cox, D.W. The effect of neuraminidase on genetic variants of alpha-1-antitrypsin. Am. J. Hum. Gen. 27:165-177, 1975

8. Bell, O.F. and Carrell, R.W. Basis for the defect in alpha-1-antitrypsin. Nature 234:410-411, 1973 (June 15)

#1043

THE COUNCIL FOR TOBACCO RESEARCH–U.S.A., INC.
110 EAST 59TH STREET
NEW YORK, N. Y. 10022
(212) 421-8885

RECEIVED
JUN 30 1975

Application for Research Grant
(Use extra pages as needed)

Date: June 25, 197

1. Principal Investigator (give title and degrees):

Hugh E. Evans, M.D.
Director, Department of Pediatrics
Jewish Hospital and Medical Center of Brooklyn

Professor of Pediatrics
Downstate Medical Center
Brooklyn, New York

2. Institution & address:

Jewish Hospital and Medical Center of Brooklyn
555 Prospect Place
Brooklyn, New York 11238

3. Department(s) where research will be done or collaboration provided:

Department of Pediatrics
Jewish Hospital and Medical Center of Brooklyn

4. Short title of study:

Relationship of non-MM phenotypes and lung disease among infants.

5. Proposed starting date: January 1, 1976

6. Estimated time to complete: Two Years

7. Brief description of specific research aims:

This study is designed to screen newborn infants for non-MM phenotypes of alpha-1-antitrypsin, to correlate the frequency, severity and type of lung disease observed in the first year and one half of life with these phenotypes, to evaluate the fertility of mothers with non-MM newborns and to contrast the biochemical and physical characteristics of the MM phenotype in newborns with those seen in infants. The questions of this investigation are: Is a newborn infant more likely to develop croup, bronchiolitis, asthma, pneumonia or other lung disease if he is of a non-MM than MM phenotype? Furthermore, are there ethnic predispositions to both the non-MM phenotype and to resultant lung disease. If non-MM phenotypic infants are at greater risk of lung disease, are there environmental control measures which could be selectively applied to mitigate these illnesses? If mothers of non-MM phenotype newborns have inherently greater fertility than those of the MM phenotype, would this have implications for family planning studies? If the MM pattern of newborn infants differs from the MM protein seen in childhood does this offer important clues regarding molecular structure?

10. Space and facilities available (when elsewhere than item 2 indicates, state location):

The newborn service at Jewish Hospital and Medical Center of Brooklyn is one of the largest in the borough with over 2700 deliveries annually. We have previously had the complete cooperation of the Department of Obstetrics, Dr. Morton Schiffer, Director and would again in the proposed study. The Pediatric Out Patient Department and In Patient units are fully staffed and equipped to carry out the proposed studies. The Loewe Laboratory has carried out the proposed tests of alpha-1-antitrypsin for the past 1½ years. Refrigerators, centrifuges, electrophors, and the usual laboratory reagents and supplies are available.

11. Additional facilities required:

None

12. Biographical sketches of investigator(s) and other professional personnel (append):

13. Publications: (five most recent and pertinent of investigator(s); append list, and provide reprints if available).

8. Brief statement of working hypothesis:

Non-MM phenotypes may play a major role in the pathogenesis of common, severe respiratory diseases of infants. This may be particularly true in crowded, environmentally adverse conditions typical of the ghetto population we serve. Furthermore, there may be ethnic determinants, as suggested in emphysema among adults. Perhaps infants with non-MM phenotypes have an imbalance between proteolytic enzymes derived from bacteria, leukocytes or alveolar macrophages and serum inhibitory capacity. Screening of newborn infants may be a practical approach to identification of those at high risk for development of subsequent lung disease. Environmental control may mitigate pulmonary disorder in such cases.

9. Details of experimental design and procedures (append extra pages as necessary)

Enrollment period: Umbilical cord sera Pi phenotyping will be obtained following each normal full term delivery at the Jewish Hospital and Medical Center of Brooklyn (JHMCB) from January 1, 1976 to July 1, 1976. Based on earlier experience we would anticipate that 1,000 infants will be included and that 80 of these will have a non-MM phenotype. Phenotyping will be done by crossed antigen-antibody electrophoresis, originally described by Fagerhol and Laurell,[1,2] or by isoelectrofocusing Quantitation of serum inhibitor will be carried out with radial immunodiffusion[3] and the antitrypsin activity test of Erlanger.[4] Each non-MM infant will be matched randomly for date of birth, sex and race with an MM newborn for purposes of subsequent follow-up.

Evaluation period: Over an 18 to 24 month interval each of the non-MM and control cases will be evaluated from a clinical point of view. They will receive their "well-baby" care in the clinics devoted to that purpose at the JHMCB. They will also be treated for all illnesses, respiratory or otherwise, and admitted to the ward as clinical judgment dictates. Every 3 months their hospital records, and the records of visits to their private physicians will be analyzed for the following:

1. Episodes of all illness.
2. Episodes of all respiratory illness.
3. Specific respiratory tract diagnosis, including chest x-rays, CBC, blood gases, bacterial culture results.
4. Hospitalizations, number, duration, discharge diagnosis; laboratory data as in #3.
5. Growth and development at age 1 and 2 years.

The data derived from each of the 2 groups will be compared to determine if there is a difference in the frequency of respiratory or other illness between the MM and the non-MM groups. Family counselling, based on medical knowledge is not possible, at present. Indeed information derived from the follow-up of non-MM infants may form the basis for such advice in the future.

question #13 (continued)

1. Evans, H.E., Mandl, I. and Glass, L. Serum Enzyme Inhibitors, Immunoglobulins and Upper Respiratory Tract Bacteria in Asthma. Am. Rev. Resp. Dis. 117:416-418, 1971 (October)

2. Mandl, I., Keller, S., Fierer, J.A. and Evans, H.E. The Role of Proteolytic Enzyme Inhibitors and Connective Tissue Proteins in the Maturation of the Lung. Harvard Conference on Respiratory Distress Syndrome. Academic Press, 99-115, 1973

3. Evans, H.E., Keller, S. and Mandl, I. Lung Tissue Elastin Composition in Newborn Infants with the Respiratory Distress Syndrome and Other Diseases. Journal of Clinical Investigation. J. Clin. Invest. 54:213-217, July, 1973

4. Fierer, J., Mandl, I. and Evans, H.E. Alpha-1-antitrypsin in the Lungs of Newborns with Respiratory Distress Syndrome. J. Ped. 85:698-701, Nov. 1974

5. Evans, H., Formaini, N. and Mandl, I. Prevalence of Pi types among newborns of different ethnic backgrounds. Protides of Biological Fluids 23rd Colloquium H.P. Peeters, ed. Pergamon Press, 1976

16. Other sources of financial support:
List financial support from all sources, including own institution, for this and related research projects.

CURRENTLY ACTIVE

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
NONE			

PENDING OR PLANNED

Title of Project	Source (give grant numbers)	Amount	Inclusive Dates
Please see letter accompanying this application.			

letter states "We plan tentatively to apply for other sources of funding but have not done so as yet. If you wish I would be glad to send copies of future requests to you."

It is understood that the investigator and institutional officers in applying for a grant have read and accept the Council's "Statement of Policy Containing Conditions and Terms Under Which Project Grants Are Made."

Principal investigator

Typed Name: Hugh E. Evans, M.D.

Signature: Hugh E. Evans M.D. Date: 6/25/75

Telephone: 212 (Area Code) 240-1000 (Number) 1776 (Extension)

Checks payable to

Jewish Hospital and Medical Center of Brooklyn

Mailing address for checks

555 Prospect Place

Brooklyn, New York 11238

Responsible officer of institution

Typed Name: Mr. Philip C. Abrams

Title: Executive Director

Signature: Philip C. Abrams Date: 6/25/75

Telephone: 212 (Area Code) 240-1000 (Number) 1201 (Extension)

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question #14b (continued)

1.	ampholines	$360
	complete set including buffer tanks cooling plates tubing leads flow meter	890
	acrylimide gel BIS (N, N^1-Methylene-bis-acrylimide) TEMED (N, N, N^1, N^1-tetraethylene-diamine) ammonium pensulfate	45
	TOTAL	$1,295
2.	sodium barbitol	200
	anti-serum	1,500
	starch	100
	Whatman #3 filter and Reeves Angel papers	120
	Tris buffer	60
	glassware	100
	pipets-disposable, nondisposable micro, 5 ml, 10 ml	200
	freezer boxes for sample storage for serum and slides	200
	TOTAL	$2,480
3.	BAPNA	50
	trypsin	120
	TOTAL	$170
4.	Radial Immunodiffusion Material	400
	TOTAL	$400

GRAND TOTAL $4,345

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